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Research report

Involvement of α7nAChR in electroacupuncture relieving neuropathic pain in the spinal cord of rat with spared nerve injury

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ABSTRACT

Alpha-7 nicotinic acetylcholine receptor (α7nAChR) was reported to be involved in the modulation of neuropathic pain. Electroacupuncture (EA) has therapeutic effects on neuropathic pain induced by nerve injury, but the underlying mechanisms remain unclear. The present study was designed to investigate whether α7nAChR participates in the relieving effects of 2 Hz EA on neuropathic pain. Paw withdrawal threshold (PWT) was measured to study the EA-mediated analgesic effect in a rat model of spared nerve injury (SNI). The spinal α7nAChR and IL-1β expression levels were determined by RT-PCR, Western blot analysis, and immunofluorescence staining. Additionally, immunofluorescence targeting the expression of CD11b, which is a molecular indicator of microglial activation. The results showed that 2 Hz EA stimulation significantly improved the expression of α7nAChR and reduced the production of IL-1β and CD11b in the spinal cord of rats with SNI-induced neuropathic pain, along with the relief of mechanical hypersensitivity after EA treatment. Moreover, intrathecal injection of alpha-bungarotoxin (α-Bgtx), a selective antagonist for α7nAChR, at the dosage of 1.0 μg/kg, not only suppressed the analgesic effect of EA in SNI rats, but also inhibited the enhancement of α7nAChR expression and the reduction of IL-1β expression induced by EA. In conclusion, our study indicated that 2 Hz EA reduces SNI-induced mechanical hypersensitivity via upregulating α7nAChR and downregulating IL-1β and CD11b in the spinal cord of SNI rats, which might be one of the mechanisms underlying its effectiveness in the neuropathic pain.

1. Introduction

Neuropathic pain induced by peripheral nerve injury is a prevalent and severe problem due to its complicated mechanism. Spinal microglia are activated after nerve injury and participate in neuropathic pain initiation and maintenance (Tsuda, 2016; Guo et al., 2007). The activated microglia together with some cytokines (i.e. IL-1β, IL-6 and TNF-α) play a fundamental role in the process of pain and induce pain hypersensitivity in the spinal cord (Tsuda, 2016; Guo et al., 2007; Watkins et al., 2001).

Previous studies have demonstrated that Alpha-7 nicotinic acetylcholine receptor (α7nAChR) mediated anti-inflammatory effects through modulating pro-inflammatory cytokines (Egea et al., 2015). This cholinergic anti-inflammatory pathway modulates the immune system through acetylcholine (ACh) that acts on α7nAChR expressed on macrophages and immune cells (Egea et al., 2015). ACh downregulates cytokine and prevents tissue damage via directly interacting with α7nAChR (Egea et al., 2015; AlSharari et al., 2013), and thus attenuates pain hypersensitivity in chronic pain.

The α7nAChR was expressed on spinal pain transmission pathways (Cordero-Erausquin et al., 2004). This provides a clue for the utility of α7nAChR agonists in the treatment of chronic pain. Abundant evidences indicated that α7nAChR plays an important role in neuropathic pain (Feuerbach et al., 2009). Moreover, α7nAChR agonists have been shown to elicit significant anti-inflammatory and anti-nociceptive effects in neuropathic pain (de Jonge and Ulloa, 2007). Taken together, these results strongly suggested a role for nicotinic pathways in the development and maintenance of neuropathic pain mechanisms, which are at least partly dependent on the α7nAChR.

Electroacupuncture (EA) has been used in China and other oriental countries in the management of many diseases including chronic pain with few side effects (Rapson et al., 2003a). Our previous study and other results have showed that EA has been effective in treating neuropathic pain (Huang et al., 2004; Chen et al., 2016; Zeng et al., 2016), however, the underlying mechanisms for the analgesic effect of EA on neuropathic pain remain unclear. Emerging evidence indicated that spinal microglia was an important target of EA in its analgesic effects related to neuropathic pain (Xu et al., 2016). 2 Hz EA-induced analgesia

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depends on the activation of different descending mechanisms, which include the spinal muscarinic mechanism (Josie et al., 2011), suggesting the involvement of cholinergic anti-inflammatory pathway in EA-induced analgesia. Furthermore, the increase of hippocampal M1 mAChR and β2 nAChR protein expression underlies the EA-mediated analgesia on SNI-induced neuropathic pain (Chen et al., 2016). These studies suggest that muscarinic and nicotinic signaling probably mediate the modulation of chronic pain induced by EA. However, it has never been thoroughly studied whether the spinal α7nAChR participates in EA-induced analgesia to neuropathic pain.

Thus, the present study was designed to investigate the effect of 2 Hz EA on pain behaviors, the expressions of spinal α7nAChR and proinflammatory cytokines (i.e. IL-1β) in SNI-induced neuropathic pain. Additionally, we conducted immunofluorescence for CD11b as the molecular indicator of microglial activation. To further explore the functions of α7nAChR in EA-mediated analgesia in SNI model, intrathecal injection of α7nAChR antagonist α-Bgtx was performed, which might extend our understanding to the mechanism of EA stimulation in the treatment of neuropathic pain.

2. Material and method

2.1. Ethics statement

In all experiments, measurements were taken to minimize pain and/or discomfort. All experiment procedures were approved by the Animal Use and Protection Committee of Gannan Medical University, according to the guidelines of the International Association for the Study of Pain.

2.2. Animals and grouping

Adult Male Sprague–Dawley (SD) rats weighing 180–220 g were purchased from Hunan SLAC Laboratory Animal Co., Ltd. (Changsha, China). The rats were housed five per cage in a temperature-controlled (22 °C–24 °C) room with a 12/12-h light–dark cycle, together with food pellets and water ad libitum. The behavioral experiments were conducted in a double blind way. Rats were randomly divided into sham, SNI and SNI + EA groups (n = 6–16). To verify the effect of α7nAChR antagonist α-Bgtx on EA-mediated analgesia in SNI neuropathic pain, additional rats were randomized into sham, SNI + Vehicle, SNI + EA, SNI + α-Bgtx + EA and SNI + α-Bgtx groups (n = 6–9).

2.3. Experiment procedure

Two different experimental procedures are shown in the schematic diagram in Fig. 1. For the first procedure, rats were divided into 3 groups, i.e. sham, SNI and SNI + EA. The sham rats suffered sham surgery, while the other groups received SNI surgery. The rats in the SNI + EA group were administered with 2 Hz EA at day 2 after the surgery and once every other day lasting for 21 days. The rats in the sham group and the SNI group were treated the same as the rats in the SNI + EA group without EA stimulation. Paw withdrawal threshold (PWT) was measured at the ipsilateral hind paws immediately following the EA stimulation on day 1, 3, 5, 7, 14 and 21 post SNI surgery. Rats from each group were sacrificed after PWT measurement on day 3, 7, 14 and 21 post SNI surgery (Fig. 1A).

In the second procedure, to further explore whether α7nAChR takes part in regulation of EA-mediated analgesia, rats were divided into 5 groups. Rats with SNI surgery were intrathecally administered once every other day with 1.0 μg/kg alpha-bungarotoxin (α-Bgtx), a selective antagonist for α7nAChR, 30 min prior to EA treatment. PWT was tested immediately after 2 Hz EA treatment, which administered once every two days lasting for 9 days. Rats from each group were sacrificed after PWT measurement on day 9 (Fig. 1 B).

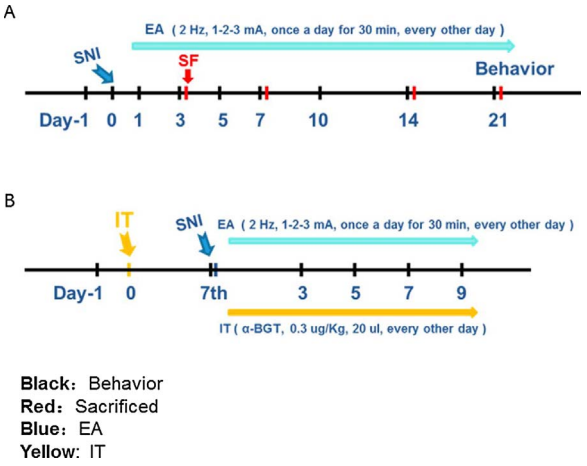

Fig. 1. The schematic diagram for two different experimental procedures. (A) PWT was measured followed by EA stimulation on day 1, 3, 5, 7, 14 and 21 after SNI surgery. Rats were sacrificed after PWT measurement on day 3, 7, 14 and 21 after SNI surgery. Rats were administered with 2 Hz EA at day 2 after the surgery and once every other day lasting for 21 days. (B) Rats with SNI surgery were intrathecally administered once every other day with 1.0 μg/kg α-Bgtx, 30 min prior to EA treatment. PWT was tested immediately after 2 Hz EA treatment with once every other day lasting for 9 days. Rats were sacrificed after PWT measurement on day 9.

2.4. Spared nerve injury (SNI) model of neuropathic pain

Following a 5-day environmental adaptation, the SNI model of neuropathic pain was established in rats according to the method described by Decosterd and Woolf (2000). Briefly, rats were anaesthetized with 1% pentobarbital sodium (50 mg/kg, i.p.), and then the left sciatic nerve and its trifurcations were exposed. The left common peroneal and tibial nerves were tightly ligated with 5.0 silk thread, and sectioned distal to the ligation with removal of 2–4 mm of the nerve stump, leaving the sural nerve intact. Muscle and skin were closed in two layers under sterile operation. Only animals that developed mechanical allodynia were used. Sham-surgery rats experienced all surgical procedures except for nerve injury.

2.5. Measurements of mechanical hypersensitivity

The sensitivity to mechanical stimuli was determined by quantifying PWT of the hind paw in response to mechanical stimulation. This processing was conducted as described previously (Zeng et al., 2016). In brief, rats were first acclimatized in individual plastic enclosures (12 × 22 × 18 cm) on a metal mesh stand for 15 min before testing. PWT was taken by a dynamic plantar aesthesiometer (Ugo Basile, 37450, Italy), which consists of a force transduction fitted with a 0.5-mm diameter polypropylene rigid tip. A probe was applied perpendicularly to the mid-plantar surface of the hind paw with an increasing pressure. The cutoff pressure was set to be 50 g and the force that caused the withdrawal response was automatically recorded by the anesthesiometer. Three measurements were taken with an interval of 5 min for the stimulation of each hind paw. Mean PWT of the three trials was obtained for each rat.

2.6. EA stimulation

Rats were restrained in specially designed holders with their hind legs and tails exposed as described in our previous report (Huang et al., 2004). Briefly, the skin of the hind legs of the rats was sterilized with 75% alcohol. Two stainless-steel needles (0.4 mm diameter, 4 mm length) were inserted into each leg. One needle was at the Zusanli acupoint (ST36), which was 5 mm lateral to the anterior tubercle of the tibia marked by a notch, and the other needle was at the Sanyinjiao acupoint (SP6), which was 3 mm proximal to the medial melleolus and